Supporting Statement
For OMB Review and Approval of

Agency for Toxic Substances and Disease Registry (ATSDR)
Anniston Community Health Survey: Follow-up and Dioxin Analyses
(ACHS-II)

Part B. Collections of Information Employing Statistical Methods

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PART B. Collections of Information Employing Statistical Methods

Background

From 2003 to 2005, the Agency for Toxic Substances and Disease Registry (ATSDR) funded the Anniston Consortium for Environmental Health Research (ACEHR), a university and community partnership charged to plan and prepare for the 2005-2007 Anniston Community Health Survey (ACHS). The ACHS employed a two-stage sampling procedure in which 3,320 households were randomly selected from a commercial list of all residential sites within the city limits. Addresses in west Anniston, the location of the former PCB manufacturing facility, were oversampled. All sampled addresses were visited by study staff: 489 were found to be vacant or nonresidential, and residents of 890 could not be contacted after multiple attempts. Contact was made with a member of each of the remaining 1,823 targeted households, and 713 declined to participate. Among the remaining 1,110 consenting households, an adult > 18 years of age was randomly selected for survey completion; 774 of those volunteered to provide a blood sample and 766 had PCB levels measured (Silverstone et al., 2012). Using the American Association for Public Opinion Research (AAPOR) methodology, the survey's response rate would be 39% (1,110/(3,320-489); http://www.aaport.org). The ACHS Cohort members had a median age of 56 years (range: 18-93 years); were largely female (70%); and either African American (46%) or white (54%). They were also mostly from the West Anniston area (84%) per the sampling plan. At the time of the ACHS, the Cohort had lived in Anniston for a median of 28 years (range: <1-79 years) (Silverstone et al., 2012).

B.1. Respondent Universe and Sampling Methods

Currently, the ATSDR, National Institutes of Health (NIH), and university investigators will conduct a follow-up to the 2005-2007 ACHS, called the ACHS-II.

- *Study Population for Recruitment:* All of the surviving original ACHS Cohort members with valid PCB measurements will be asked to participate in the ACHS-II in order to enroll a sample of 500 respondents.

- Recruitment will be conducted by University of Alabama at Birmingham (UAB) and Calhoun County Health Department (CCHD) study staff. Study offices for appointments will be located in Anniston for a period of approximately 8 to 12 weeks. Designated secured space will be available for recruitment activities, reception, obtaining informed consent, enrollment, obtaining biological samples, and conducting interviews.

- The UAB investigator has retained information in identifiable form (IIF) and the last known contact information from the 766 members of the original ACHS Cohort with PCB measurements (IIF more fully described in Section A.1). Uses of the ACHS and the updated IIF are graphically presented in Attachment 3 (Overview of Data Collection System, Information Flow Chart).

- For planning purposes, a preliminary search of the Social Security Death Index (SSDI), of internet address databases, and of population data from the U.S. Census (USCB, 2011) was performed to estimate the number of the ACHS Cohort members likely to be deceased or to have moved from the study area (shown in Attachment 3 – Overview of Data Collection System, Information Flow Chart).

    - As the ACHS included subjects up to 93 years of age, it is expected that some have died since 2005-2007. All-cause mortality for Calhoun County is estimated to be 1.2 percent, a rate that has remained stable between 2005-2007 (ADPH/ARHA, 2009) and 2009 (Auburn University, 2011). As of January 20, 2012, 66 of the ACHS Cohort are classified as likely to be deceased, based on matches on *name*, and *date of birth* between the ACHS Cohort and the SSDI.

    - For the remaining individuals assumed to be living, the list of 700 *names* were run through two Internet-based reference databases (411.com and ReferenceUSA.com) in an attempt to confirm current matches on their name, approximate age, original street address and original telephone number. When these reference databases indicated the individuals had moved since the ACHS, updated address, updated zipcode, and updated telephone number were obtained. As of January 20, 2012, UAB obtained current contact information on 488 Cohort members, and the whereabouts of 212 Cohort members are unknown. As the population in the City of Anniston has declined by 4.8 percent between the 2000 and the 2010 Censuses (USCB, 2011). It is estimated that approximately 35 ACHS Cohort members are no longer living in the study area.

- Because Social Security Numbers (SSNs) were not collected and the matches with the SSDI and internet based databases are not confirmatory, a study information and recruitment package (Attachments 3.1 &3.2) will be mailed to eligible ACHS Cohort members. The confirmation of change of address or undeliverable notice will be received from the United States Postal Service (USPS).

- Recruitment progress will be monitored and the number of mailouts will be adjusted to achieve the enrollment goals. UAB study staff will work with the West Anniston Foundation to further update addresses, zip codes, and

telephone numbers in the ACHS Cohort Contact List. The Foundation is a socially connected non-profit 501(c)(3) public charity corporation with strong ties to the residents and former residents of West Anniston. Efforts to maximize response rates are discussed below. Final response rates will be obtained at the end of the enrollment period.

- *Eligibility:* For the ACHS-II, eligibility is restricted to the fixed ACHS Cohort of 766 members with serum PCB analytic measures.
    - Pregnant women will be excluded from the study *(as greater than minimal risk)*.
    - Persons currently serving a criminal sentence or under house arrest will be excluded *(protected class under 45 CFR 46)*.
    - Eligible Cohort members, who are not willing or able to provide a blood specimen, are allowed and encouraged to participate in the questionnaire and clinical assessments. Collecting a partial blood sample is also allowed if the subject cannot provide 125-ml.
    - Eligible Cohort members who request or require a home interview and blood draw must reside within a one-hour drive from the study offices. Exceptions may be granted to this requirement on case-by-case basis if resources allow.

Sample size estimation: The proposed sample size (n=500) was based on the ability to answer three research questions: 1) to detect differences in mean PCB congener levels between baseline and the ACHS-II; 2) to detect differences in prevalent cases of health outcomes between PCB exposure groups in the ACHS-II (cross-sectional) and 3) to detect differences in incident cases of health outcomes between PCB exposure groups since baseline (prospective). Sample size of 500 was also proposed to maximize the resources available for the study. As detailed below, a smaller sample size would be sufficient for most exposure assessment analyses and analyses of prevalent health outcomes (the main goals of the study).

For detecting differences in mean PCB congener levels, we assumed a simple exponential decay model to describe the expected mechanism for human metabolism and excretion of PCBs based on the work of Seegal et al. (2011). In this model and with simplifying assumptions, $C_{(t)}$ represents the predicted mean ACHS-II serum concentration, $C_{(0)}$ represents the mean ACHS I serum PCB concentration, K represents the decay constant, and t is the time interval between studies. Given $K = \ln(2)/hl$, it follows that $C_{(t)}$ can be estimated.

We selected representative congeners with low (PCB 118, four chlorines), moderate (PCB 153, six chlorines), and high (PCB 206, nine chlorines) chlorination. PCB 118 is also mono-*ortho* substituted and dioxin-like, PCB 153 di-*ortho* substituted and non-dioxin-like. PCB 206 is a tetra-

ortho substituted congener. The three congeners represent a spectrum of PCBs with different chlorination patterns, toxicities, and shorter to longer half-lives (Hansen, 1998).

Based on the work of Knobeloch et al. (2009), for all three congeners, we assumed: 1) that the metabolism and elimination of each PCB congener follows an exponential decay model; 2) that additional individual exposure to PCB congener since baseline is minimal; and 3) that the change in mean PCB congener concentration is lognormally distributed. We also assumed a half-life of 14 years under the null hypothesis. We assumed *t*, the time since baseline to be 7 years and an alternative hypothesis of a half-life of 20 years. Sample size and power estimations were performed using one-sided one-sample t-tests, 80 percent power, and $\alpha = 0.05$. Assumptions and proofs are further described in Attachment 7.

Table 1. Mean ACHS PCB congener concentrations and associated sample size required seven years later for the ACHS-II.

PCB Congener	Mean ACHS PCB Concentration $C_{(0)}$, in ng/g lipid	Standard Deviation of ACHS PCB Concentration	Sample Size at 80% Power and α=0.05
118	70	177	420
153	218	409	232
206	40	98	395

Therefore, under these assumptions, we have sufficient power to detect a difference in mean PCB concentrations for low, moderate, and highly chlorinated PCB congeners between two time points. Exposure assessment analyses that compare PCB congeners measurements between the two study time points thus can be meaningfully performed with sample sizes of 400 or even 200 respondents (i.e. PCB 153). If only 100 respondents were enrolled the power to detect statistically significant difference would be substantially reduced. Although not taken into account in the above decay model assumptions, we recognize that factors such as the body composition and weight (or weight change) may substantially alter elimination rates for PCBs (Chevrier et al., 2000; Ritter et al., 2011).

For detecting differences in health outcomes by PCB exposure, it has been demonstrated that the original study, the ACHS, had enough power to detect associations between PCB levels and prevalence of hypertension, high blood pressure, and diabetes (Goncharov et al., 2010, 2011; Silverstone et al., 2012). Adding an average of six years of follow-up should further increase the number of prevalent cases of diabetes, heart disease, and other chronic diseases that tend to increase with age. Of critical interest for the prospective component of the follow-up study is the ability to examine incident cases of disease since baseline. As an example, we present the estimation of incident cases of diabetes and the power calculation estimate to detect differences in exposure variables (i.e. PCBs) between diabetics and non-diabetics.

There were 205 prevalent cases of diabetes (Silverstone et al., 2012) identified in the first Anniston study (27% of 766). Since then, we estimate that about 66 Cohort members are deceased. We make the simplifying assumption of similar death rates between diabetics and non-diabetics. As 27 percent already had diabetes at baseline, approximately 511 surviving Cohort members would still be at risk of developing diabetes after baseline. We plan to enroll 500 respondents of the surviving 700 Cohort members (71.4%) in the follow-up study. Therefore, we assume that proportionately 365 of 500 ACHS-II respondents would require assessment for incident diabetes since baseline.

The national age adjusted incidence rates for diabetes for 18- to 79-year-olds ranged between 9.8/1,000 and 13.0/1,000 per year for Black (average 11.1/1,000) and between 7.0/1,000 and 8.0 /1,000 per year for White (average 7.8/1,000) population for 2005-2010 period (CDC, 2012). Assuming our sample is half White and half African American we could use combined average rate of 9.5 /1,000 per year for those without diabetes (State of Alabama age adjusted incidence rate for the same period was slightly higher and ranged between 9.8 and 11.3/1,000; CDC, 2012). However, of those without diabetes in the original study (n=561), 169 subjects were found to have impaired fasting glucose and classified as pre-diabetics. These persons would likely have a higher rate of developing diabetes, estimated at 50/1,000 to 100/1,000 a year (Inzucchi and Sherwin, 2008). Proportionately, out of 365 persons without diabetes, 110 would likely be pre-diabetic and would have estimated to develop about 33 incident diabetes cases (using a conservative estimate of 50/1,000 a year). Combined with an estimated 16 cases of incident diabetes for normoglycemic individuals (255 out of 365, average rate of 9.5/1,000 as derived above) we would estimate to detect a total of 49 incident diabetes cases in the period of 2006-2013 in this sample of Anniston population consisting of normoglycemic and pre-diabetic individuals (Attachment 7).

In respect to being able to detect the difference in PCBs levels, we reported that levels in normoglycemic individuals were 6.31 ng/g wet weight and 7.71 ng/g wet weight for diabetics (geometric means; sum of 35 PCB congeners, Silverstone et al., 2012). For pre-diabetics, the PCB levels were similar to normoglycemic individuals (6.16 ng/g wet weight). For this estimate, we used log-transformed mean levels of the sum of PCBs and standard deviations in the two sample t-test power analyses (Machin et al., 1997); common mean total PCB level was used for all non-diabetics. Applying the above assumptions, we would have 87% power to detect statistically significant difference at alpha=0.05 level of confidence in PCB levels between 49 new cases of diabetes and 316 non-diabetics (Attachment 7).

Using the same assumptions, the power to detect significant differences between incident cases of diabetes and non-diabetics by enrolling 400 respondents would be 79% (39 diabetics, 253 non-diabetics), 68% when enrolling 300 respondents (29 diabetics, 190 non-diabetics), and 54% when enrolling 200 (20 diabetics, 126 non-diabetics) respondents (Table 2). It is challenging to conduct follow-up studies of environmental exposures, recent publications on diabetes and PCBs only included 36 diabetes cases each (Turyk et al., 2009, Lee et al. 2012). Repeated PCB measurements were only obtained in Turyk et al. study.

Table 2. Power to detect differences in PCB congener levels in incident and prevalent cases of diabetes.

No. Enrolled	Incident Diabetes		Prevalent Diabetes*	
	No. with Diabetes Mellitus /No. Non-diabetic	Power	No. with Diabetes Mellitus /No. Non-diabetic	Power
200	20/126	54%	40/160	77%
300	29/190	68%	60/240	90%
400	39/253	79%	80/320	96%
500	49/316	87%	100/400	98%

*Estimated prevalence of diabetes – 20%.

For prevalent diabetes, the achieved power would be higher due to a higher number of included prevalent diabetes cases even with the relatively smaller sample size. For modeling purposes and to provide conservative estimates, we assumed that the prevalence of diabetes would decrease from 27% in 2005-2007 Anniston sample to 20% or 10% in the planned follow-up study sample (decreasing the power to detect associations). Other assumptions were the same as in estimating power based on differences in PCB levels in the incidence power calculations. The power to detect differences when enrolling 400 respondents with the diabetes prevalence of 20% and 10% (80 and 40 diabetes cases, respectively) would be 96% and 81%, respectively. When enrolling 300 respondents, the power would be 90% and 71%, respectively. Only 51% power would be achieved if 100 respondents enrolled in the study with the diabetes prevalence of 20% (data using 10% diabetes prevalence not shown in Table 2).

The provided numbers are only estimates based on the available data; the actual number of incident diabetes cases and PCB levels may be different. Where available, we used conservative estimates to avoid overestimating diabetes cases. With the relatively high prevalence of chronic disease in the Anniston sample, a sample size smaller than 500 would still provide data that could detect statistically significant associations between diabetes or heart disease and PCBs at sufficient power.

The study investigators acknowledge that the sample size of 500 represents a high estimate of how many respondents may actually be recruited from the pool of about 700 surviving cohort members (response rate of 71%). It is possible that a further 35 may have moved out of Anniston. In order to recruit a sample of 500 respondents, we assume up to 160 of the Cohort may be lost to follow-up or may refuse to participate, and approximately 5 will be currently ineligible (e.g., pregnant or serving criminal sentences) (Attachment 3). To recruit 500 respondents out of 600, an 83% response rate would be required. The methods to maximize the recruiting potential are described in the following section. As detailed above, study results would be useful and meaningful if less than 500 respondents were recruited. Further details on assumptions, mathematical proofs, and calculations are provided in Attachment 7.

B.2. Procedures for the Collection of Information

Data Collection

A graphical overview of the information collection process is included in Attachment 3. The CCHD study office will be the designated center for obtaining biological samples and conducting interviews. Alternatively, a satellite office will be set up at the West Anniston Foundation facility. For interviews by home visit, the data collection will follow procedures for data and biological sample collection consistent with the office visits. In these cases, a team consisting of an interviewer and a public health nurse will be sent to the household.

Data collection steps: Study staff will be trained on the goals and purposes of informed consent, interview, and blood specimen collection methods, and on proper documentation of data collection procedures. Staff will receive NIH training on Human Subjects Protection and sign a confidentiality agreement prior to contact with interested recruits and enrolled respondents. Trained staff from the CCHD will attend dedicated telephone lines to respond to questions and to address concerns from interested recruits, enrolled respondents, and the public. Interested recruits will be asked to attend their appointment in at least an eight-hour fasting state; therefore, most recruits are expected to schedule appointments in the early morning. The steps of the data collection will include:

1. Check-in procedures;
2. Data collection station assignments;
3. Exit procedures; and
4. Provision of a gift card as a token of appreciation for participation.

Completion of each data collection step will be documented by the administering staff on the hardcopy Appointment Tracking Form (Attachment 3.7), which will be hand carried by the recruit-respondent from station to station as assigned. Trained study staff at each station will document the completion of each step from check-in to the provision of gift cards. As part of the exit procedures, the respondent will sign this form to document receiving the gift card. This hardcopy form will be stored with the respondent's signed Informed Consent Form (Attachment 3.8) in locked files and in secure rooms.

Check-in procedures: Study coordinators or assigned staff will check-in each arriving recruit to:

1. Obtain informed consent and enrollment;
2. Update contact information;
3. Assess current medications; and
4. Make station assignments to maximize workflow.

Informed consent and enrollment: Before any data collection can begin, trained study staff will review the hardcopy Informed Consent Form (Attachment 3.8) with the interested recruit to explain the purpose of the study and to obtain written informed consent for both the survey

and the collection of blood specimens. The informed consent includes a description of study procedures and risks and benefits of participation. A study factsheet will inform respondents of the chemical tests and clinical outcomes to be measured (Attachment 3.2 – Study Factsheet). Emphasis will be placed on the voluntary nature of participation. The interviewer will answer any questions the interested recruit has prior to obtaining signatures.

The risks of participation in this study are minimal (defined in 45 CFR 46.110). This study plans for a one-time 125-ml volume of fasting blood to be collected. This volume is less than 25 percent of the amount of blood that the Red Cross collects during a routine donation (about 450 ml -– average adult male has 5.7 liters, average adult female has 4.3 liters of blood). The body manufactures blood continuously and replaces this volume of blood within 24 hours (CDC, 2005). To assure minimal risks, pregnant women and persons who are sentenced or under house arrest will be excluded from the study. The risk of discomfort, infection or hematoma will be minimized by using trained nurses or phlebotomists. Diabetics who are prescribed medications or insulin for control of their blood sugars will be given special instructions for their blood draw. They will be advised to continue their meal and medication plan as prescribed. As fasting blood draws are preferred, trained study staff will offered appointments as early as possible. If the diabetic respondent is unable to fast prior to the blood draw, they will be requested to select meals that are fat-free or low-fat, if possible. After the blood draw, the respondent will be offered a small snack, thereby allowing monitoring of acute events due to phlebotomy.

The main benefit from participating in this study will be to help the Anniston community and scientific researchers to better understand how chemical exposures might be related to human health. The schedule and budgeting for analytical testing will take place at multiple laboratories over multiple years; therefore, there will be limited personal benefit from receiving test results such as lead, cadmium, mercury, fasting glucose, lipid profiles, thyroid hormones, and immune titers because of the length of time for analysis to be completed.

Contact information update: After written informed consent is obtained, the recruit will become an enrolled ACHS-II respondent. Each enrolled respondent will be asked to verify and update his or her current contact information for results reporting in a CAPI (Attachment 3.9 – Update Contact Information Form).

Current medication list: Each respondent will be asked to bring in all of his or her current prescription medications in the zipped plastic bag previously mailed in the Appointment Packet. Trained study staff will review the containers and record prescription medications, over-the-counter medications, supplements, vitamins, and herbal remedies on the Medications List in a CAPI (Attachment 3.10). This will help to validate respondents' responses about their health condition(s). Respondents will be reminded to gather all their usual medications for the past two weeks for the appointment (Attachment 3.6).

Station assignments: Study staff will instruct the respondent to carry a hardcopy form from station to station to document data collection progress (Attachment 3.14 - Appointment Tracking Form). Assigned study staff will record responses, measurements, and disposition codes at each station until the interview and blood draw are completed. Station assignments include:

1. Body and clinical measurements
2. Fasting blood specimen collection
3. Questionnaire

The order of station assignments may be altered to minimize waiting time or to accommodate the fatigue or preference of the respondent; however, if performed immediately together, resting blood pressure must be measured prior to venipuncture.

Body and clinical measurements: Trained study staff will perform the body and clinical measurements. These include the respondent's height, weight, BMI (calculated), waist circumference (girth), hip circumference, and blood pressure (Attachment 3.11). The measured blood pressure level is subject to biological and observer variability. The purpose of a specific measurement protocol, or training and certifications of technicians and of ongoing quality control is to minimize variability due to known exogenous factors and to reduce imprecision and biases in measurement.

Resting blood pressure, height, weight, and waist circumference may be measured in any order, but blood pressure should be obtained after the subject has been in the seated position for at least five minutes. Blood pressure will be measured before venipuncture if the activities are scheduled consecutively. Trained study staff will record the measurements in the Body and Blood Pressure Measures Form (Attachment 3.11) in a CAPI. Referral decisions for abnormal blood pressure will be documented on this form.

Blood specimen collection: As a safety precaution, trained staff will quickly re-screen each respondent in a CAPI for conditions that would prevent a respondent from safely giving blood: hemophilia, chemotherapy in the past four weeks, and skin or arm lesions/disorders at the blood draw site (Attachment 3.12). Respondents with those conditions will be allowed to take part in questionnaire and body measurements of the study.

Before the blood draw, the staff will also re-assess and record the current pregnancy status for women less than 60 years of age. Upon discovery, pregnant women will be excluded from the study. The use of diabetes medication or insulin, and fasting status will also be noted. Fasting diabetic respondents who use insulin will be given priority appointments for their blood draw. If the respondent is not fasting, the time of the last meal and items consumed will be noted.

Next, trained nurses or phlebotomists will draw 125-ml (10 tablespoons) of blood using standard venipuncture techniques. Trained study staff will record the phlebotomy result on the

Blood Draw Form (Attachment 3.12). If a person is unable to provide 125-ml of blood, a smaller amount is allowed (50-ml would be desirable) and will be documented for those who weigh less than 110 lbs., only 50-ml of blood will be collected. Common adverse events from blood draws include bruising, bleeding, and fainting. No serious adverse events are anticipated in drawing this volume of blood. Light snacks will be provided following blood collection.

Questionnaire: Trained study staff will administer the questionnaire as a CAPI (Attachments 3.13b & 3.13c.

The ACHS-II questionnaire is divided into 11 main sections (Attachment 3.13b): 1) residential history; 2) (demographic) background; 3) general health and chronic health conditions; 4) physical activity; 5) health behaviors; 6) diet; 7) health care access; 8) women's health history; 9) men's health history; 10) children's health history; and 11) work history. Depending on skip patterns in the CAPI, not all respondents will be asked the four supplemental forms for: 1) children's health; 2) female health; 3) male health; and 4) local foods. To increase recall and comprehension and to reduce time burden, the Interviewer's Booklet will be used as a visual aid for the respondent (Attachment 3.13c).

Using the baseline ACHS questionnaire as a template, the current questionnaire has retained the content of many ACHS modules for consistency, with revisions for clarity and brevity where applicable to accommodate the follow-up design. Questions were rephrased to collect supplemental information since the 2005-2007 data was collected. ACHS questions are retained (original - O), modified (M), or developed (new – N) for the ACHS-II Questionnaire. To minimize the respondent's time in the study, some of the baseline information that will not change over time or that were not informative, will not be recollected for the ACHS-II. These similarities and changes between the ACHS and the ACHS-II questionnaires are indexed in Attachment 3.13a.

The ACHS questionnaire was previously tested for reliability and validity. For the ACHS-II, the modified questionnaire was pilot tested among study staff for comprehension, flow, and ease of administration.

B.3. Methods to Maximize Response Rates and Deal with Non-response

Study Roll Out: To increase community and former participants' awareness and to maximize response rates, announcements in advance of the start of the study will be released through local news media, meetings with community representatives, and/or public meetings and forums. The same outlets may be used for public messages for ongoing community outreach and study information dissemination (Attachment 8).

***Mailout 1 - Recruitment Information Packet*:** Efforts to inform eligible respondents about the upcoming study include an introductory recruitment mailout to encourage participation. The Recruitment Information Packet will be sent by certified mail to all former ACHS Cohort

members with PCB measurements. This packet will contain a cover letter of invitation (Attachment 3.1), and a study factsheet (Attachment 3.2). As part of the invitation to participate, these materials will explain the purpose and description of the study and the selection criteria for inclusion in the study. It will also explain the potential risks and benefits, the location of the study, the sponsoring agencies, and the person to contact for further information. The total time in the study and plans for offering a gift card to respondents will also be included. All study staff will sign confidentiality agreement form to be able to handle respondents' data collected in the study.

Recruitment Information Packets will be sent in approximate batches of 100 packets per week to schedule a manageable flow of interviews on any day. This schedule of mailouts will be adjusted as needed to complete the data collection as quickly as possible. As part of the recruitment tracking procedures, return services will be requested from the USPS. Undeliverable packets will be returned with the new address information affixed and without forwarding service. This will allow study staff to update address information prior to resending another Recruitment Information Packet to updated Anniston addresses. All changes of address will be documented in the Update Contact Information Form (Attachment 3.9).

***Recruitment telephone calls*:** If the selected recruit does not voluntarily respond within two weeks of the recruitment mailout, trained study staff will begin telephone recruitment. The selected recruit will be called up to 10 times at different times of the day and on different days of the week. Once successful contact is achieved, study staff will administer the Recruitment Telephone Script in a CATI (Attachment 3.3). All calling attempts, the level of effort, the disposition codes, and any actions taken will be recorded on the Recruitment Tracking Form (Attachment 3.7) for each recruit.

Study staff will be trained to maximize conversion of undecided and "soft-refusals", and to locate those recruits who cannot be contacted. This may include offering alternative dates or study office, assistance with transportation or home visits to undecided and soft-refusals who cannot or are not willing to visit study office. For those recruits who do not have phones or current numbers listed, study staff will employ alternative contact recruitment methods, including consultations with the West Anniston Foundation to locate the recruit for current addresses and telephone numbers, or to attempt home visits to personally deliver the Recruitment Information Packets. All alternative contact attempts and disposition codes will be recorded on the Recruitment Tracking Form (Attachment 3.7).

For those selected recruits who are hard refusals, study staff will administer a Survey for Refusals (Attachment 3.4) in a CATI as an aid for non-response analysis.

***Mailout 2 - Appointment Packet*:** Selected recruits who are eligible and willing to participate, will be scheduled an appointment at the study offices, or alternatively at a home visit for those who are unable or unwilling to attend an office visit. A toll-free telephone line will also be offered to encourage respondents to schedule appointments at their convenience. Once the

appointment is scheduled, study staff will mail an Appointment Packet containing an Appointment Reminder Card (Attachment 3.5), the Informed Consent Form (Attachment 3.8), a zipped plastic bag to bring in all medications to the appointment. An advance copy of the Informed Consent Form will provide an extra opportunity for the interested recruit to read and more fully understand his or her rights in the study and to ask any questions before the scheduled appointment.

***Reminder telephone* calls:** Study staff will give the interested recruit a reminder telephone call one to two days before the scheduled appointment in a CATI (Attachment 3.6 – Reminder Telephone Script). The study protocol will provide the flexibility to schedule or re-schedule office or home visits. Respondents who are unable or unwilling to come to the study office will be offered an in-home appointment by trained study staff to complete the study. Respondents who request or require a home interview and blood draw must reside within a one-hour drive from the study offices. Missed appointments will be followed up by at least five contact attempts by study staff for rescheduling in order to maximize the number of completed appointments (Attachment 3.7 – Recruitment Tracking Form).

Recruitment conditions specific to the citizens of Anniston: After decades of controversy, the Anniston community is very aware of the unique nature of its PCB exposure. Four major litigation efforts, three ATSDR health consultations, four U.S EPA consent degrees for soil clean up, and participation in the ACHS have made it clear that Anniston is an environmental justice community. Environmental justice is defined as the "fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies (U.S. EPA. See http://www.epa.gov/environmentaljustice/).) With half of the original study group being African-American and the many decades it took to bring the issue of PCB contamination to light, this community is still deeply affected by feelings of anger, betrayal and distrust towards authorities and government (Dr. Rhoda Johnson, presentation at the community meeting, April 2008). Monsanto never revealed the potential for human exposure to PCBs, the level of environmental contamination in Anniston, or ways to mitigate or prevent human exposure until litigation started. Involvement of government agencies also began in earnest only after the whole issue was widely covered in media.

Historically, the community feels that they were never properly compensated for exposure to toxic substances. These Cohort members may also believe that their participation in studies, which may provide new scientific information but does not directly or immediately benefit their personal health, may be unwarranted. More importantly, the design of the follow-up study does not allow for replacement participants from the general public; if the former study participants refuse to be involved, we have no way of replacing these individuals. Thus, to encourage as many ACHS participants as possible to return for this follow-up study, we propose a gif card amount higher than in most studies that draw participants from the general population.

As a token of thanks for respondents' interest, and for their willingness to provide 125-ml of blood, a $150 gift card will be offered to each respondent who takes part in both the interview and the blood draw. If the respondent must (such as for a safety precaution against a blood draw) or chooses to take part only in the interview, a $75 gift card will be offered; likewise, for choosing to take part only in the blood draw, a $75 gift card will be offered. Payments will be documented on the Appointment Tracking Form (Attachment 3.14). A detailed discussion of proposed tokens of appreciation is provided in Section A.9.

Characteristic of non-response/non-response analyses plan: Regarding the original ACHS, we conducted some basic analyses of non-response. The table in Attachment 9 shows characteristics of ACHS non-participants, participants who completed only the interview, participants who completed the interview and had PCBs measured, as well as comparative information about residents of Anniston and of Calhoun County.

The non-participants shown in the table (n=737) are persons from 713 households that were contacted but declined to participate. They were asked about their age, race, sex, residence in west or east Anniston, and why they did not wish to participate. Sampling in the original study was based on housing units located in west Anniston (proximate to soil and water PCBs contamination) and east Anniston (remote from contamination). As shown in the table, it is difficult to compare these demographic characteristics because of missing data for non-participants (22%-60%). We did not conduct statistical tests whether these proportions were statistically significantly different. Generally, the proportion of males and Whites was similar between participants and non-participants. Per sampling design, the majority of contacted individuals were from west Anniston. This proportion was higher in participants (84%) than in non-participants (72%). The most frequent reasons for refusal were "Not interested" (41%), "No response given" (35%), "Too busy" (12%), and "Health issues" (5.7%) (Attachment 9).

Participants' race and age distributions were similar to those of the city of Anniston, with a lower proportion of those younger than 40 years among participants; the proportion of participants who had completed high school was 5% to 7% lower than in Anniston overall. As mentioned earlier, 70% of the participants were female. There were small differences in reported demographic characteristics between those participants who completed only the interview (n=1,110) and those who completed the interview and had PCB measurements (n=765) (Attachment 9).

Not shown in the table are the 890 could-not-contact households. The only information available on them is their address and the reason for noncontact (e.g. n=734 "Not home", n=22 "Dog", n=86 "Gate locked", and n=35 "Other restricted entry").

For the follow-up study non-response analyses, we have detailed information on the 766 persons who responded to the first study and gave blood samples, as well as for those who only completed the first study interview. For persons not known to be dead, we plan to conduct an analysis (using logit models and/or survival analysis) of their participation in the follow-up study

taking into account demographics, health characteristics at the time of the original study (e.g. general assessment of health, high blood pressure, diabetes, etc.) and their housing unit's proximity to contamination. For those known to be dead, we will conduct an analysis of the probability of death in relation to the same types of variables.

B.4. Tests of Procedures or Methods to be Undertaken

Statistical analyses will be performed by ATSDR staff in collaboration with the NIH. Outside investigators may be involved in statistical analyses of study data. Descriptive statistics (including means, medians, and percentiles) will be calculated to identify the presence and distribution of analytes in the target population. Univariate analysis will be performed to determine the influence of exposure variables that might affect serum/blood levels of analytes (e.g., local fish species consumed, frequency or amount consumed, age, gender, and race). If the distributions of residuals in linear regression analyses are not normal, they will be transformed for further analysis. Multivariate regression modeling will be performed for each target analyte to determine factors associated with the increased serum/blood levels.

Prevalence estimates will be calculated using standard statistical procedures for each analyte found in the target population. Results of chemical analyses may be compared to data reported in the CDC National Reports on Human Exposure to Environmental Chemicals, which is based on National Health and Nutrition Examination Survey (NHANES) data (CDC, 2009; http://www.cdc.gov/exposurereport/index.html). The analytic measures of lipophilic PCDD, PCDF, and PCBs will be expressed on both a wet weight basis and on a per lipid weight basis to allow these results to be compared to other studies of varying assumptions and analytical and reporting methods (Longnecker, 2001).

Logistic regression models for health outcomes of interest will be used to adjust for potential confounders collected in the study. The follow-up design will be used to calculate and present changes in levels of previously measured PCB congeners and clinical analytes. The incidence of disease since the first examination can be enumerated and difference based on exposure levels described. Repeated measures analyses for continuous variable adjusted for changes in weight or BMI or other pertinent covariates are also planned. To further examine the shape of the dose–response curves, a generalized additive model (GAM) (Hastie and Tibshirani, 1990) may be fitted using a cubic regression spline for the ΣPCBs or other exposure variables.

PCBs will be included in the analyses as 'total' PCBs (the sum of 35 PCB congeners), individual PCB congeners, and subsets of PCBs. Subsets of PCBs based on structure and function will be summed as follows: estrogenic congeners 44, 49, 66, 74, 99, 110, and 128 (DeCastro et al., 2006); mono-*ortho* congeners 28, 66, 74, 105, 118, 156, 157, 167, and 189; mono-*ortho* dioxin toxic equivalents (TEQ) 105, 118, 156, 157, 167, and 189 (Van den Berg et al., 2006); di-, tri-, and tetra-*ortho* congeners (combined) 44, 49, 52, 87, 99, 101, 110, 128, 138+158, 146, 153,

170, 172, 180, 194, 149, 151, 177, 178, 183, 187, 195, 196+203, 199, 206, and 209; and the ryanodine-like congeners 52, 101, 149, 151, 170, 180, 183, and 187 (activate ryanodine receptors at < 1 µM) (Pessah et al. 2006). In addition, we will evaluate the possible association between dichlorodiphenyldichloroethylene (DDE) and other organochlorine pesticides with study outcomes of interest. Sum of organochlorine pesticides have also been used in statistical analyses. Dioxins, dibenzofurans, and coplanar PCBs will be analyzed individually and as sums of their respective TEQs. Total dioxin TEQ (sum of individual PCDD/DF/co-PCBs/mono-*ortho* PCBs) will be calculated and contrasted with health outcomes of interest using 2005 World Health Organization toxic equivalency factors (WHO TEFs, Van den Berg et al., 2006).

Testing of secondary hypotheses, sum of PBDE congeners, individual PBDE congeners, individual heavy metals, and pro-inflammatory cytokines will be included in different regression models. Confounding of associations with PCBs and dioxins (from the primary hypotheses) will be tested as well as potential effect modification. Major risk factors for health outcomes of interest collected from questionnaire data and respondents' measurements will also be evaluated. Interaction terms between exposure variables and age, race, and sex will be constructed and evaluated in the regression models. Cytokines and inflammatory factors could also be evaluated as modifiers of primary health outcomes.

Confounding will be assessed in a series of models that include exposure variable (e.g. PCBs) and one of the established risk factors for outcome variable or potential confounders. Confounding is defined as a > 10% change in the β-coefficient and will evaluated by comparing point estimates for the exposure-outcome associations with and without the potential confounder. Effect modification will be investigated using variables indicating the product of the potential effect modifier with the exposure, and by stratification of regression models by the potential effect modifier.

B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

The following table presents the ACHS-II research team.

Table 3. Investigators and Key Study Personnel

Name	Affiliation and Title	Phone	Email
PRINCIPAL INVESTIGATORS			
Marian Pavuk, MD, PhD	ATSDR – Principal Investigator; Senior Epidemiologist	770-488-3671	fsh8@cdc.gov
Stephen Mennemeyer, PhD	UAB – Co-Principal Investigator; Professor, School of Public Health	205-975-8965	smenneme@uab.edu
CO-INVESTIGATORS			
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Paul Wolff, PhD	UAB - Research Coordinator	205-975-8050	pwolff@ms.soph.uab.edu
Paul Jung, MD, MPH, MBA, MA	NIH – Chief of Staff, NIEHS	919-541-7758	paul.jung@nih.gov
Mike Sanders, PhD, DABT	NIH – Director, NCI Laboratory of Toxicology and Toxicokinetics	919-541-1872	sander10@mail.nih.gov
Stephanie I. Davis, MSPH	ATSDR - Epidemiologist	770-488-3676	sgd8@cdc.gov
Michael Lewin, MS	ATSDR - Mathematical Statistician	770-488-3812	mdl0@cdc.gov
COLLABORATORS (UNDER CONTRACT)			
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Allen Silverstone, PhD	SUNY Upstate Medical Center, Syracuse, NY – Professor	315-464-5871	silversa@upstate.edu
Carol Spencer, PhD	University of Southern California, Los Angeles, CA – Laboratory Director	626-993-2809	cspencer@usc.edu
CONSULTANTS			
Scott Bartell, PhD	University of California Irvine, Irvine, CA – Associate Professor	949-444-3545	sbartell@uci.edu
Christie Shelton, PhD	Jacksonville State University, Jacksonville, AL – Associate Dean, College of Nursing	256-782-8427	cshelton@jsu.edu

B.5.1 Study design and data collection plan:

The study design, enrollment and data collection plan was developed as a collaborative process between all investigators and contract collaborators listed in Table 3. Specifically, the lead on the design of data collection plan was Marian Pavuk (ATSDR), the lead for data collection will be Stephen Mennemeyer (UAB). Additional advisors are listed below.

Name	Title	Phone	Email
COMMUNITY INTERACTION ADVISORS			
Shirley Carter	Community Activist	256-525-2986	msabccarter@yahoo.com
Kay Beard	West Anniston Foundation - Executive Director	256-238-8476	kaybeardal@bellsouth.net

Additional consultations with CDC, university, and medical center laboratories included the subject matter experts listed in Table 3 and as described in Section A.8.

B.5.2 Questionnaire design

The leads on the questionnaire design were Marian Pavuk (ATSDR), Stephen Mennemeyer (UAB), Linda Birnbaum, Mike Sanders, Paul Jung (NIH), and Stephanie Davis (ATSDR). CATI and CAPI development and testing were performed by SRU staff under the supervision of Paul Wolff and Andy Rucks. Additional consultations with Alabama state agencies included the following subject matter experts as described in Section A.8.

Name	Title	Phone	Email
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ALABAMA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT (ADEM)			
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B.5.4 Data Management

UAB offers its established information and research technology services through its Survey Research Unit (SRU – see http://www.uab.edu/cores/survey-research-unit). A formally designated UAB Service Center, the SRU works with university investigators as well as state and national groups. Services include technical assistance in survey design and sampling methods, provision of computer assisted telephone survey interviews, and field survey research. With 40 CATI stations, four supervisor stations and a trained IRB-certified, staff of 80-100, at any given time, the SRU is equipped to conduct large-scale computer assisted telephone surveys. Supervisor stations have the ability to monitor telephone calls and view the computer entries made by the interviewers for quality control. All stations are connected to the main server which houses the survey software (Sawtooth/Ci3) and CATI system. All stations have back-up power supplies and are password-protected. The SRU also provides services for completing surveys in-person, by mail or fax and offers data entry services. A state-of-the-art system is in-place to conduct web based surveys and to create survey instruments that can be scanned. In addition, salaried positions for doctoral students have been established to assist users with data analysis and manuscript preparation.

Name	Title	Phone	Email
DATA MANAGEMENT			
Andrew Rucks, PhD	UAB Survey Research Unit - Director	(205) 975-8967	ARucks@ms.soph.uab.edu

B.5.5 Data Analysis

Data analyses will be conducted collaboratively with Marian Pavuk, Michael Lewin (ATSDR), and Linda Birnbaum (NIH) as leads. Sample size estimation and power calculations were developed by Michael Lewin; Stephanie Davis, and Marian Pavuk (ATSDR). Additional consultants on data analysis included the following:

Name	Title	Phone	Email
DATA ANALYSIS			
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